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FURTHER EXPERIMENTS IN THE DESTRUCTION OF FLY LARVÆ IN HORSE MANURE.

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INTRODUCTION.

The results reported in this bulletin are a continuation of the investigation dealt with in Bulletin No. 118, United States Department of Agriculture, inaugurated for the purpose of finding a substance that would destroy the larvæ of the house fly in their principal breeding place, namely, horse manure, without injuring the bacteria or reducing in any way the fertilizing value of the manure (Cook, Hutchison, and Scales, 1914). The work was conducted in cooperation by the Bureaus of Entomology, Chemistry, and Plant Industry at Arlington, Va., and New Orleans, La. The bacteriological work at New Orleans was done by Dr. William Seemann, dean of the Tulane School of Tropical Medicine, and thanks are due him for his cooperation. The entomological work at New Orleans was done by Mr. E. R. Barber, scientific assistant, Bureau of Entomology.

In Bulletin No. 118 it was suggested that manure be treated with borax immediately on removal from the barn in order to destroy the eggs and maggots of the house fly, and that borax be applied at the rate of 0.62 pound per 8 bushels, or 10 cubic feet, of manure. As large quantities of manure are used by truck growers, it was thought advisable to include in that bulletin a warning as to the possible injurious action of large applications of borax-treated manure on plants. For the same reason it seemed desirable to find some volatile or other organic substance which would be effective as a larvicide, but without possible toxic action on vegetation. Largely with this object in view, the investigation was continued during 1914. The larvicidal value of some inorganic substances was also tested.

Borax may be used with advantage for the treatment of outhouses, public dumps, and refuse piles of all kinds, cracks and crevices, floors of stables, and any accumulation of organic material which offers a

favorable place for the deposition of eggs. A treatment two or three times a week ought to suffice for all these cases except where large quantities of organic material are added, when the borax application should be made immediately, using the same quantity as in the treatment of horse manure. The best results are always obtained when the borax is applied in solution daily, as it is effective against the eggs and the maggots during their feeding period. (Table V, series 52, G and H.) Borax probably has no effect on the pupæ or adult flies.

GENERAL PLAN OF EXPERIMENTAL WORK.

CAGE EXPERIMENTS.

The plan of the work was the same as that outlined in Bulletin No. 118, and in addition a few experiments were carried out in concrete pits. New cages were constructed for the experiments at Arlington. In order to prevent the escape of larvæ by migration, the galvanized-iron pans in the cages in which the manure was placed were made 2 feet deep, and the small openings in the bottom of the pans through which the water drained off were covered with fine wire gauze. The legs were made 8 inches high to facilitate the removal of any larvæ which might get into the drip pans.

The manure was sprinkled in three layers by putting 2 bushels of manure in the cage and applying 2½ gallons of the solution. This was repeated in the second layer of 2 bushels. Finally the remaining 4 bushels were added and the last 5 gallons of the solution applied. When a chemical was applied in dry condition it was scattered over the surface of the manure, which was also treated in three layers, and 10 gallons of water were afterwards added. The manure in the control cages was sprinkled with water equal to the volume of the solutions used. The flies which were caught in the traps attached to the top of the cages were chloroformed and counted, and at the end of each experiment a comparison of the total number was made, and from these counts an index of the effectiveness of the chemical was obtained. Only fresh manure was used in the experiments, and every effort was made to provide for an even distribution of fly eggs and larvæ. That it was impossible to secure an equal infestation in all cages is evident from a comparison of the fly counts from the control cages.

OPEN-PILE EXPERIMENTS.

A few open-pile experiments were carried out at Arlington on the same plan as during the previous year. The most important open-pile experiments were conducted at New Orleans during November and December, 1914. In most of the New Orleans open-pile experiments 4 bushels of manure were sprinkled with 5 gallons of solution daily, and this was repeated four times, making a total pile of 16 bushels treated with 20 gallons of solution. The total number of

pupæ in each pile was counted about eight days after the last treatment. A sample of 200 pupæ from each pile was kept in the laboratory, and the percentage of emergence determined. From these data the apparent larvicidal effect was calculated. For some of the New Orleans experiments, cages (Pl. I, fig. 1, p. 16) were constructed to cover the piles, and instead of counting the number of pupæ the flies were allowed to emerge and were caught in traps attached to the tops of the cages. Temperatures and samples for analysis were taken through the armholes in the sides of the cages. Soil was banked against the base of the cages to prevent the escape of maggots and flies.

Chemical and bacteriological analyses were made of samples of manure from most of the cage and open-pile experiments.

SAMPLING FOR CHEMICAL AND BACTERIOLOGICAL ANALYSES.

It is very evident that a manure pile with the unequal distribution and great variation of its physical and chemical constituents will necessarily be exceedingly difficult to sample, especially to secure from it a few hundred grams which will be thoroughly representative. An attempt was made to secure representative samples by taking equal portions of manure from three different parts of the pile, spreading them on a clean sheet of paper, and finely dividing and thoroughly mixing them. When the material appeared quite uniform the sample was quartered. One quarter was then cut into half-centimeter lengths with clean shears. The straw or shavings were cut with the other material. When this operation was completed the sample was again thoroughly mixed. For chemical analysis the material was twice passed through a grinder. Both bacteriological and chemical examinations were made on the same sample. As the bacterial content of manure is very high, no attempt was made to work under absolutely sterile conditions, because the contamination arising from ordinary handling of the material was of no importance when compared with the great number of organisms present. However, precautions were taken to prevent excessive contamination by using clean paper, shears, etc., for each sample.

BACTERIOLOGICAL EXAMINATION.

Two 10-gram samples of the manure, prepared as just described, were taken for each bacteriological determination. One of the 10-gram samples was dried at 100° C. for one hour to determine the percentage of solids. The other sample was brushed into a 2-liter flask containing 1 liter of sterile water. The flask was then vigorously shaken for five minutes and again, after a five minute interval, for three minutes. A 1-c. c. sample was then withdrawn and run into 100 c. c. of sterile water. Five dilutions were prepared, ranging from 1 part in 10,000 to 1 part in 100,000,000. A duplicate series of Petri dishes was then prepared from these dilutions and standard beef agar.

After five days' incubation at 28 to 30° C. the plates were counted. The average counts of the duplicate plates were taken and converted into equivalents for 1 gram of dry manure by the use of the figures obtained from the duplicate 10-gram samples that had been dried at 100° C. The results obtained by plating on the standard beef agar are comparative and serve to show the germicidal action of the chemicals on the majority of the bacteria present in the manure. Dr. Seemann, in the work at New Orleans, used a medium prepared from manure water, but the counts were practically the same as those on beef agar.

CHEMICAL EXAMINATION.

The manure samples were analyzed for solids, ash, ammonia, and nitrogen, using the methods of the Association of Agricultural Chemists (Wiley, 1908). The total nitrogen determinations were made by the Nitrogen Laboratory of the Bureau of Chemistry. The results obtained by the magnesium-oxid distillation method for ammonia, which are not reported in the table, although much higher, showed the same general tendencies as the figures obtained on the water extracts.

Water extracts were prepared from each sample by taking 25 grams of the finely divided manure and adding 500 c. c. of distilled water, allowing them to stand for one hour, with occasional shaking. The solutions were filtered, and the following determinations were made: Water-soluble nitrogen, ammonia, nitrites, nitrates, and reaction.

Ammonia was extracted by the Folin and Macallum (1912) aeration method and nesslerized. Nitrites were determined with the sulphanilic acid reagent, and nitrates by the reduction method with aluminum foil (American Public Health Association, Laboratory Section, 1912). Nitrites and nitrates were not usually found in the samples examined, because the manure had not stood sufficiently long. The reaction was determined by taking 20 c. c. of the water extract, diluting with 200 c. c. of carbon-dioxid free water, and titrating with twentieth normal acid, using alizarin red as indicator.

GENERAL ACCOUNT OF SUBSTANCES USED.

Representatives of two groups of substances were tested during the season's work, namely, (1) inorganic and (2) organic, including volatile and nonvolatile substances and some plant material. These substances are arranged in alphabetical order in the respective groups.

INORGANIC SUBSTANCES.

Of the inorganic substances, arsenical dip, chlorid of lime, Epsom salts, lime-sulphur, and sulphuric acid in three concentrations were used.

ARSENICAL DIP.

Arsenical dip, which is extensively used in the West and Southwest to kill ticks on cattle and sheep, was prepared according to the directions given in U. S. Department of Agriculture Farmers' Bulletin No. 603 (Chapin, 1914). This solution was used in three cage experiments, namely, full strength, and diluted 1 to 1 and 1 to 3 (Table I, Series 67, A, B, and C).

TABLE I.—*Destruction of fly larvæ in horse manure. Results with arsenical dip, paradichlorobenzene, and pyridine; cage experiments at Arlington, Va., 1914.*

Series.	Treatment of 8 bushels of manure, using 10 gallons of liquid.	Flies emerged.	Apparent larvicidal effect.	Number of bacteria per 1 gram manure, calculated to dry weight.	Manure, total nitrogen.	Water extract.		
						Alkalinity, N/20 HCl per 100 c. c. (5 grams of manure).	Water-soluble nitrogen.	N as NH_3, Folin method.
		Number.	*Per cent of control average.*	*Millions.*	*Per cent.*	*C. c.*	*Per cent of total nitrogen.*	*Per cent of total nitrogen.*
67 A	Arsenical dip (strong)	67	87	1,648	0.421	10.50	33.63	3.5
67 B	Arsenical dip (1–1)	57	89	1,944	.337	5.75	29.97	4.4
67 C	Arsenical dip (1–3)	131	74	2,459	.547	6.50	33.82	3.7
70 A	Control (water only)	826	....	1,636	.625	6.40	27.84	....
70 B	.....do	382	....	1,043	.463	8.75	41.25	7.1
70 C	.....do	275	....	843	.337	8.40	26.70	4.7
	Control average	494	....	....	....	....	....	....
78 A	Pyridine, 1–100	37	63	634	.540	8.90	31.11	3.0
78 B	Pyridine, 1–500	135	0	798	.540	8.65	31.11	3.7
81 A	Control (water only)	53	....	1,035	.611	9.90	26.68	1.9
81 B	.....do	252	....	1,282	.639	11.15	36.93	2.4
81 C	.....do	96	....	....	....	....	....	....
	Control average	134	....	....	....	....	....	....
104 A	Pyridine, 1–100	3	99+	209	.470	4.75	28.72	7.02
104 B	.....do	2	99+	128	.449	4.50	33.85	2.45
106 A	Control (water only)	4,489	....	19.6	.572	4.25	25.00	2.10
106 B	.....do	1,936	....	27.1	.561	4.65	26.02	1.41
	Control average	3,212	....	....	....	....	....	....
79 A	Para-dichlorobenzene, ½ pound to 10 gallons water	10	93	905	.793	13.15	36.07	4.8
79 B	.....do	70	50	502	.505	11.65	35.44	4.6
	(For control see 81, A, B, and C, above.)							
98 A	Para-dichlorobenzene, 1 pound to 10 gallons water	11	97	....	....	....	....	....
98 B	.....do	68	78	....	....	....	....	....
99 A	Control (water only)	157	....	283	.898	11.40	25.61	2.34
99 B	.....do	687	....	165	.653	10.75	24.19	4.13
99 C	.....do	77	....	157	.533	8.15	27.39	2.44
	Control average	307	....	....	....	....	....	....

The two stronger concentrations killed about 88 per cent of the maggots and the 1–3 solution about 75 per cent. The weaker strengths apparently exerted a slight stimulating action on the bacteria, and the full-strength dip showed no bactericidal action in this one test. The chemical data showed no marked differences except for the increased alkalinity where the dip was applied in full strength, and this, no doubt, was due to the sodium carbonate in the dip.

CHLORID OF LIME.

Chlorid of lime has been used extensively as a disinfectant and has been tested by Dr. Howard, who found that 1 pound applied to 8 quarts of manure killed practically all the larvæ, but one-fourth of a pound to 8 quarts was not sufficient (Howard, 1911). In our experiments with smaller amounts, namely three-fourths of a pound, $1\frac{1}{2}$ pounds, and 3 pounds to 8 bushels and the addition of 10 gallons of water, negative results were obtained. The well-known action of chlorid of lime in driving off the ammonia from manure, the probable toxic effect on bacteria, and the irritating action of the liberated chlorin, as well as the high cost of the substance in quantities sufficient to kill fly larvæ, precludes its use for this purpose.

EPSOM SALTS.

Epsom salts was applied in three cage experiments, using respectively 1, 2, and 4 pounds to 10 gallons of water. In no case was any larvicidal effect noticed. No chemical or bacteriological examinations were made.

LIME-SULPHUR.

Lime-sulphur was used again this year in dilutions of 1 to 10, 1 to 20, and 1 to 30. No larvicidal effect was seen, and since it failed to kill the maggots, no chemical or bacteriological analyses were made.

SULPHURIC ACID.

Sulphuric acid was used in 1, 2, and 3 per cent solutions. Two cage experiments with each concentration were carried out. Practically no larvicidal effects were shown in any of the experiments. Sufficient alkaline substances and organic material were present in the manure to combine with the acid in the 1 per cent and 2 per cent solutions, consequently no injurious action on the bacteria resulted. No counts were made where the 3 per cent solutions were applied. When the 3 per cent applications were made the alkaline reaction of the manure was markedly reduced and the percentage of ammonia, in terms of the total nitrogen, was increased from three to four times that of the control. The 1 per cent and 2 per cent solutions had no apparent action on the manure as determined by the chemical results.

ORGANIC SUBSTANCES.

As the application to the soil of manure containing inorganic substances is likely to produce harmful effects on plants, due to a slight excess of the toxic element in the soil, it seemed desirable to investigate the larvicidal action of various organic substances, both volatile and nonvolatile. The volatile substances would produce

a true partial sterilization, as has been shown by several investigators (cf. Russel and Buddin, 1913, and the recent article by the latter, Buddin, 1914), while the nonvolatile substances would be finally decomposed in the soil, and, therefore, have no permanent injurious effect. Organic substances, when added to the soil, may be attacked by various members of the soil flora, as bacteria and filamentous fungi, which either destroy the substances entirely or form compounds that may be utilized by other organisms which are of value in maintaining the fertility of the soil or may be utilized directly by the plants themselves.

ANILINE.

Aniline ($C_6H_5NH_2$), which is extensively used in the preparation of dyes, contains 15 per cent of nitrogen and costs about 60 cents per quart. This substance was tested in cage experiments at Arlington, Va., using dilutions of 1 to 50, 1 to 100, and 1 to 200.

TABLE II.—*Destruction of fly larvæ in horse manure. Results with aniline and nitrobenzene; cage experiments, Arlington, Va., 1914.*

Series.	Treatment of 8 bushels of manure, using 10 gallons of liquid.	Flies emerged.	Apparent larvicidal effect.	Number of bacteria per 1 gram manure, calculated to dry weight.	Manure, total nitrogen.	Water extract.		
						Alkalinity, N/20 HCl per 100 c. c. (5 grams of manure).	Water-soluble nitrogen.	N as NH_3, Folin method.
		Number.	*Per cent of control average.*	*Millions.*	*Per cent.*	*C. c.*	*Per cent of total nitrogen.*	*Per cent of total nitrogen.*
94 A	Aniline, 1-50	4	98	264	0.526	8.25	41.63	7.98
94 B	Aniline, 1-100	11	97	392	.632	8.65	33.70	4.60
94 C	Aniline, 1-200	25	80	....	....	....	....	....
99 A	Control (water only)	157	....	283	.898	11.40	25.61	2.34
99 B	....do	687	....	165	.653	10.75	24.19	4.13
99 C	....do	77	....	157	.533	8.15	27.39	2.44
	Control average	307	....	....	....	....	....	....
100 A	Aniline, 1-200	17	99+	....	.618	4.15	25.08	1.40
100 B	....do	28	99	....	.674	6.40	29.08	1.63
106 A	Control (water only)	4,489	....	19.6	.572	4.25	25.00	2.10
106 B	....do	1,936	....	27.1	.561	4.65	26.02	1.41
	Control average	3,212	....	....	....	....	....	....
95 A	Nitrobenzene emulsion (3¼ pounds nitrobenzene and ¼ pound fish-oil soap to 10 gallons water)	0	100	551	.547	9.75	34.92	9.14
95 B	....do	1	99+	667	.526	8.10	40.50	7.41
95 C	Same as foregoing, diluted 1-3	115	63	....	....	....	....	....
99 A	Control (water only)	157	....	283	.898	11.40	25.61	2.34
99 B	....do	687	....	165	.653	10.75	24.19	4.13
99 C	....do	77	....	157	.533	8.15	27.39	2.44
	Control average	307	....	....	....	....	....	....
101 A	Nitrobenzene (1.67 pounds nitrobenzene and ⅓ pound fish-oil soap to 10 gallons water) undiluted	4	99+	....	.565	6.40	34.69	8.32
101 B	....do	0	100	323	.449	5.40	34.96	9.13
101 C	Same as foregoing, diluted, 1-1	5	99+	....	....	....	....	....
106 A	Control (water only)	4,489	....	19.6	.572	4.25	25.00	2.10
106 B	....do	1,936	....	27.1	.561	4.65	26.02	1.41
	Control average	3,212	....	....	....	....	....	....

The results given in Table II show a 97 and 98 per cent larvicidal action with the two stronger solutions, and in the case of the 1 to 200 dilution two cages showed a 99 per cent and one an 80 per cent larvicidal action. The 1 to 50 and 1 to 100 strengths showed an increased number of bacteria. The amount of water-soluble nitrogen and ammonia is noticeably increased in the manure treated with the strongest solution of aniline, with no apparent action on the bacteria. The increases of the water-soluble nitrogen and ammonia are probably due to nitrogen in the aniline added. Aniline, among other substances, was tried in some cage experiments at New Orleans, the results of which are given in Table III.

TABLE III.—*Destruction of fly larvæ in horse manure. Results with aniline, hellebore, and nitrobenzene; cage experiments at New Orleans, La., 1914.*

Series.		Treatment of 8 bushels of manure, using 10 gallons of liquid.	Flies emerged.	Larvæ in drip pans.
			Number.	*Number.*
107	A	Aniline, 1-200	3,738	2,380
	B	Aniline, 1-400	6,030	12,853
	C	.....do	5,070	9,856
108	A	Hellebore, ground, ½ pound to 10 gallons water	1,122	4,034
	B	.....do	820	4,567
	C	Hellebore, ground, ¼ pound to 10 gallons water	1,130	1,285
	D	.....do	874	1,930
109	A	Nitrobenzene, 1 pound, and ½ pound fish-oil soap to 10 gallons water	3,390	2,985
	B	.....do	1,692	3,647
	C	Nitrobenzene, ½ pound, and ¼ pound fish-oil soap to 10 gallons water	1,319	2,042
110	A	Control (water only)	1,085	560
	B	.....do	5,888	4,835
111	A	Aniline, 1-200	5,076	4,462
	B	Aniline, 1-400	12,667	8,296
	C	.....do	12,309	19,675
112	A	Hellebore, ground, ½ pound to 10 gallons water	11,803	10,794
	B	.....do	17,197	8,179
	C	Hellebore, ground, ¼ pound to 10 gallons water	20,067	9,315
	D	.....do	17,838	268
113	A	Nitrobenzene, 1 pound, and ½ pound fish-oil soap to 10 gallons water	2,395	10,132
	B	.....do	12	211
	C	Nitrobenzene, ½ pound, and ¼ pound fish-oil soap to 10 gallons water	9,678	11,165
114	A	Control (water only)	19,366	11,419
	B	.....do	18,838	10,726

The cages were the ones used the previous year and, as the figures show, large numbers of larvæ escaped. Apparently these results show a low larvicidal effect of aniline, but since the condition of the cages was unsatisfactory they should not be compared with the others, especially as they are the only ones, either in cages or open piles, where poor results with aniline were obtained.

Open-pile experiments at New Orleans, using a 1 to 200 dilution of aniline, killed 85 and 99 per cent of the fly maggots (Table IV, series 46, A and B); 1 to 400 dilutions in two piles gave 79 and 83 per cent larvicidal effect (Table V, series 41, C and D); and two piles treated with aniline, 1 to 500, showed an 84 per cent destruction of larvæ (Table V, series 49, A and B).

TABLE IV.—*Destruction of fly larvæ in horse manure. Results with aniline, hellebore, nitrobenzene, and pyridine; open-pile experiments, New Orleans, La., 1914.*

Series.		Treatment: 5 gallons liquid to 4 bushels manure; 4 treatments each, except series 44 and 45.	Pupæ found.	Emergence from sample of 200 pupæ.	Apparent larvicidal effect.	No. of bacteria per 1 gram manure calculated to dry weight.	Manure. Total nitrogen.	Water extract. Alkalinity, N/20 HCl per 100 c. c. (5 grams of manure).	Water extract. Water-soluble nitrogen.	Water extract. N as NH_3, Folin method.
			Number.	*Per cent.*	*Per cent of control.*	*Millions.*	*Per cent.*	*C. c.*	*Per cent of total nitrogen.*	*Per cent of total nitrogen.*
	A	Hellebore, ground, ½ pound to 10 gallons water	43,335	....	58.9	56.5	0.358	5.00	25.14	13.99
	B	.....do	40,046	....	62.0	24.2	.421	4.75	28.02	7.84
[1] 44	C	Hellebore, ground, ¼ pound to 10 gallons water	58,021	....	44.9	382	.379	5.50	31.14	13.98
	D	.....do	77,056	....	26.8	1,170	.379	4.65	29.55	12.40
	E	Control (water only)	105,196	....			.386	5.00	26.16	15.54
	A	Hellebore, ground, ⅜ pound to 10 gallons water	[2] 49,311	....	30.9	590	.400	4.00	15.50	7.50
	B	.....do	[2] 41,148	....	42.3	1,940	.400	3.90	15.50	5.50
[1] 45	C	Hellebore, ground, ¼ pound to 10 gallons water	[2] 60,107	....	15.9	1,000	.477	4.25	13.00	5.45
	D	.....do	[2] 36,201	....	49.3	440	.435	4.15	14.25	8.28
	E	Control (water only)	[2] 71,305	....	....	880	.379	4.25	13.18	6.07
	A	Aniline, 1–200	28,439	32.5	85.0	2,990	.407	6.00	41.28	16.21
	B	.....do	6,460	13.5	99.4	77	.470	6.25	35.74	17.02
46	C	Hellebore, ground, ⅜ pound to 10 gallons water	34,104	80.0	55.8	2,140	.456	4.75	29.60	11.18
	D	.....do	56,718	37.0	66.0	1,150	.435	5.25	33.56	15.63
	E	Control (water only)	61,714	93.0	....	625	.407	5.25	38.57	16.46
	A	Nitrobenzene, ¼ pound, and fish-oil soap, ½ pound, to 10 gallons water	4,140	62.5	89.4	1,240	.428	7.00	27.57	16.35
	B	.....do	7,927	56.5	81.5	533	.351	5.25	25.64	10.25
47	C	Hellebore, ground, ⅜ pound to 10 gallons water	13,770	53.0	70.0	967	.337	4.25	23.44	10.98
	D	.....do	12,640	57.5	70.2	1,780	.442	4.25	20.36	7.92
	E	Control (water only)	24,312	95.5	....	2,750	.365	5.65	27.94	12.06
	A	Pyridine, 1–1,500	122,907	82.5	8.3	484	.512	5.50	32.81	12.30
	B	.....do	76,705	75.5	47.6	750	.491	7.25	34.22	15.27
48	C	Hellebore, ground, ½ pound to 10 gallons water	49,602	84.0	62.4	1,780	.547	6.40	31.08	15.54
	D	.....do	77,431	80.0	44.0	1,330	.449	4.90	47.44	7.80
	E	Control (water only)	110,470	96.5	....	....	.505	7.25	35.64	13.86
	A	Hellebore, powdered, ½ pound to 10 gallons of water (alkaloids, 0.24 per cent)[3]	787	65.0	89.7	97.2	.393	5.65	30.00	7.89
	B	Hellebore, powdered, ½ pound to 10 gallons of water (alkaloids, 0.26 per cent)[3]	811	68.0	88.9	60.2	.400	3.90	22.50	10.00
53	C	Hellebore, powdered, ½ pound to 10 gallons of water (alkaloids, 0.41 per cent)[3]	596	72.0	91.3	64.0	.400	6.25	26.75	11.75
	D	.....do.[3]	965	60.0	88.3	97.0	.379	5.25	28.23	13.98
	E	Control (water only)	4,942	91.0	....	80.7	.400	4.25	25.25	12.50

[1] In series 44 and 45 there were 3 applications made; the first at the rate of 10 gallons to 8 bushels, the second and third at the rate of 5 gallons to 4 bushels.
[2] Number of flies emerged.
[3] Nitrites and nitrates present.

TABLE V.—*Destruction of fly larvæ in horse manure. Results with aniline, hellebore, nitrobenzene, and pyridine; open-pile experiments, New Orleans, La., 1914.*

Series.		Treatment: 5 gallons liquid added to 4 bushels of manure; 4 applications.	Pupæ found.	Emergence from sample of 200 pupæ.	Apparent larvicidal effect.
			Number.	*Per cent*	*Per cent of control.*
41	A	Pyridine, 1-500	4,756		98.6
	B	.....do	1,948		99+
	C	Aniline, 1-400	70,642		79.4
	D	.....do	56,651		83.5
	E	Control	342,771		
42	A	Nitrobenzene, 1 pound and $\frac{1}{2}$ pound fish-oil soap to 10 gallons water	96,053		75.0
	B	.....do	92,771		76.0
	C	Nitrobenzene, $\frac{1}{2}$ pound and $\frac{1}{4}$ pound fish-oil soap to 10 gallons water	74,636		80.6
	D	.....do	92,954		76.0
	E	Control	385,403		
43	A	Hellebore, ground, $\frac{1}{2}$ pound to 10 gallons water	564		99+
	B	.....do	772		99+
	C	Hellebore, ground, $\frac{1}{4}$ pound to 10 gallons water	13,265		95
	D	.....do	17,526		93.7
	E	Control	273,520		
49	A	Aniline, 1-500	1,707	67.0	84.2
	B	.....do	1,603	74.0	83.6
	C	Hellebore, powdered, $\frac{1}{2}$ pound to 10 gallons water	502	52.0	96.4
	D	.....do	162	43.0	99.0
	E	Control (water only)	7,202		
50	A	Hellebore, powdered, $\frac{1}{2}$ pound to 10 gallons water	398	49	97.8
	B	.....do	293	49	98.4
	C	.....do	449	40.5	98.0
	D	.....do	334	40.5	98.5
	E	Control (water only)	8,866	93	
51	A	Hellebore, powdered, $\frac{1}{2}$ pound to 10 gallons water	7	23.8	99+
	B	.....do	14	23.8	99+
	C	Hellebore, powdered, $\frac{1}{4}$ pound to 10 gallons water	12,294	71.0	80.0
	D	.....do	24,233	71.0	60.0
	E	Control (water only)	43,733	95	
52	A	Nitrobenzene, $\frac{1}{4}$ pound and 1 pound fish-oil soap to 10 gallons water	3,036	63	93.4
	B	.....do	3,415	63	92.8
	C	Pyretheum, powdered, $\frac{1}{2}$ pound to 10 gallons water	24,346	95.5	0
	D	.....do	31,379	95.5	0
	E	Hellebore, powdered, $\frac{3}{8}$ pound to 10 gallons water	7,694	95	75.5
	F	.....do	5,725	95	81.8
	G	Borax, powdered, 1 pound to 10 gallons water	1,390	8.5	99+
	H	.....do	953	8.5	99+
	L	Control (water only)	29,831	98.5	

As only one duplicate set of open piles treated with aniline 1 to 200 was examined, the data are not sufficient on which to base any conclusion as to its effect on the bacteriological and chemical composition of the manure. Aniline in all open-pile experiments showed a high larvicidal efficiency even in the dilution of 1 to 500, but it should be handled with care because of its possible toxic effects.

BETA NAPHTHOL.

Beta naphthol was tried in three cage experiments, using solutions containing 0.1 pound, 0.33 pound, and 1 pound, respectively. Where the 1-pound application was made only 11 per cent of the maggots were killed, and in the other two cases the results were negative. No bacteriological or chemical examinations were made of the treated manure.

CRESYLIC ACID.

Cresylic acid, which is prepared from coal tar, was tried in dilutions of 1 to 20, 1 to 40, and 1 to 80 in cage experiments, but it was without action on the maggots. Bacteriological and chemical analyses were made of the manure treated with the 1 to 20 strength, and the bacteria were reduced 90 per cent. The alkalinity of the manure was reduced, and a slight increase in ammonia over that of the controls was found.

PARA-DICHLOROBENZENE.

Para-dichlorobenzene was employed in two sets of cage experiments, using one-half pound and 1 pound to 8 bushels of manure. The substance was ground and scattered over the manure, and water added. As shown (Table I, series 79, A and B, and 98, A and B), the apparent larvicidal effect varied greatly, the one-half pound strength indicating a 50 and 93 per cent action and the 1 pound indicating 78 and 97 per cent effectiveness. The bacterial counts and chemical analyses where the one-half pound applications were made showed only slight effects from this treatment. No analyses of the manure in the cages treated with the 1-pound applications were made. Para-dichlorobenzene was tried in one pit experiment. The pits were of concrete with inside measurements of 9 by 6 by 2 feet. Thirty-two bushels of manure were placed in the pits, and the manure in one pit was treated at the rate of 0.5 pound to 8 bushels. The other pit was untreated. From the former 403 flies emerged, and from the latter, 484. The larvicidal action was, therefore, apparently about 16 per cent. Duckett (1915) has found this substance to be an effective fumigant against various household insects and those affecting stored products.

FORMALDEHYDE.

Further experiments with formaldehyde were performed, using 1 to 6, 1 to 8, and 1 to 10 dilutions of the commercial 40 per cent formalin in water. The 1 to 8 and 1 to 10 strengths showed no larvicidal effects, and the action of the 1 to 6 solution on the maggots was slight (17 per cent). The bacteriological and chemical results showed the same general tendencies as those found last year, an increased number of bacteria and a reduction of the alkalinity being observed. As the cost of formaldehyde is high, and as strong solutions are required to kill the maggots, the use of this substance is not practical for this purpose.

NITROBENZENE.

Nitrobenzene ($C_6H_5NO_2$), commercially known as oil of mirbane, costs 20 cents per pound and contains 11.4 per cent of nitrogen. The vapors of this liquid are poisonous. Two sets of cage experiments at

Arlington were carried out with emulsions of this substance and fish-oil soap. The strength of these emulsions and the results obtained are given in Table II, series 95 and 101. The larvicidal results, with the exception of series 95, C, were good. The emulsions apparently produced a considerable increase in numbers of the bacteria. An increase of water-soluble nitrogen and ammonia was obtained in all the treated samples. Some further experiments at New Orleans, using the cages which were employed in 1913, gave poor results, as many larvæ escaped (Table III, series 109 and 113). The results of six open-pile experiments are recorded in Table V (series 42, A, B, C, and D, and 52, A and B), and the data for two additional open-pile experiments are given in Table IV (series 47, A and B). Bacteriological and chemical results are given in connection with the last two experiments. The duplicate samples indicate that there was a slight reduction in the number of bacteria and that there was no apparent effect on the manure as shown by the chemical data. All the experiments indicate satisfactory larvicidal results, but the best were those obtained with the largest quantity of fish-oil soap, namely, 1 pound (Table V, series 52, A and B), which killed 93 per cent of the larvæ. This fact suggests that the fish-oil soap is an important constituent of this larvicidal mixture.

OXALIC ACID.

Four cage experiments were carried out with oxalic acid, using 1 and 2 pounds to 10 gallons of water. One experiment with 1 pound gave negative larvicidal results, while the results from three experiments using 2 pounds were as follows: 3 per cent, 60 per cent, and 80 per cent. In the one sample of manure analyzed the bacterial count was reduced, and the ammonia was increased over the control.

PYRIDINE.

Pyridine (C_5H_5N), which is prepared commercially from coal tar, and is also obtained from the distillation of bone oil, is alkaline, contains 17.75 per cent nitrogen, and costs about $1 per pound. This liquid was used in three cage experiments in dilutions of 1 to 100 and in one cage experiment in a dilution of 1 to 500. The results of the cage experiments are shown in Table I (Series 78 and 104). The larvicidal efficiency of the 1 to 100 dilutions was 63 per cent for one cage and 99 per cent for the other two. The 1 to 500 dilutions showed no apparent effect. No consistent action on the bacteria is evident, and the water-soluble nitrogen and the ammonia from the treated samples are higher than from the controls.

In open-pile experiments at New Orleans (Table V, Series 41, A and B) pyridine 1 to 500 was used twice, giving an apparent larvicidal

effect of 99 per cent. Pyridine was used in two open-pile experiments in a dilution of 1 to 1,500 (Table IV, Series 48, A and B), and 8 and 47 per cent of the larvæ were killed.

It is impossible satisfactorily to explain the differences in the larvicidal efficiency of the pyridine in the cage and open-pile experiments where the 1 to 500 dilutions were employed. Different samples of pyridine were used in these two tests, and as the conditions are very different at Arlington and New Orleans the exact larvicidal value of the 1 to 500 dilutions is uncertain. As the 1 to 1,500 is the only dilution that is practical from a cost point of view and the larvicidal effect of this strength was low, this substance is hardly thought to be worthy of further consideration as a larvicide. The extremely disagreeable odor as well as the toxicity of pyridine makes its use in this work objectionable.

PLANT MATERIAL TESTED.

In looking for substances of an organic nature it seemed advisable to test material from several common plants and weeds, especial attention being given to those that are very abundant and therefore cheap.

Dr. Alsberg suggested the use of plants containing saponin, corn cockle being named as a waste product containing considerable amounts of this compound. Agave, a saponin-containing plant growing abundantly in Texas and Florida, was obtained by Mr. W. D. Hunter and was tried in two experiments. Other plant material, some of which contains alkaloids, were also included in the investigation, namely, "blackleaf 40" (an extract of tobacco), larkspur, hellebore, ox-eye daisy, pyrethrum, and stramonium.

Corn cockle.—Corn cockle (*Agrostemma githago*) is present in wheat screenings. The screenings used in this work contained about 43 per cent of corn cockle, and hæmolytic tests [1] showed the presence of considerable saponin. The screenings were ground and then extracted with water for 12 hours. Nine cage experiments at Arlington, using extracts of the screenings containing from 0.3 of a pound to 5 pounds per 10 gallons, were tried, and the highest apparent larvicidal action was 49 per cent. These results varied markedly, and in certain cases no larvicidal effect was obtained. Many bacteriological and chemical analyses showed no change in the number of organisms or the composition of the manure.

Agave.—The roots of several agave or soapweed plants (*Agave lecheguilla*) were macerated and water extracts prepared. Two and one-half pounds of the finely divided roots were extracted for 12

[1] The hæmolytic tests were made by Dr. C. S. Smith, of the Bureau of Chemistry.

hours in 10 gallons of water. This extract was used in two cage experiments and showed a larvicidal action of 82 and 84 per cent. The manure was unaffected chemically, and the bacterial count on one of these samples was considerably higher than the control counts.

"Blackleaf 40."—"Blackleaf 40," an extract of tobacco (*Nicotiana tabacum*), containing 40 per cent of nicotine sulphate, is used to a considerable extent as an insecticide, and it seemed worth while to test its effect on fly larvæ. It was tried in three cage experiments at Arlington, Va., diluted 1 to 50, 1 to 250, and 1 to 500. In none of these cases did it show any larvicidal action.

Larkspur.—Ground seeds of larkspur (Delphinium) were tested, using solutions prepared by treating 1 pound of the ground seeds with 10 gallons of 1 per cent sulphuric acid and allowing them to extract for 12 hours. The extract was applied undiluted, diluted 1 to 5, and diluted 1 to 15. The apparent larvicidal effect varied from 57 to 90 per cent. The bacteria were not affected by the application of the undiluted extract, and the only change in the manure noted was a decrease of alkalinity due to the acid present in the extract added.

Stramonium.—A sulphuric-acid extract of the ground leaves of stramonium (*Datura stramonium*) was prepared by mixing 1 pound of the ground dried leaves with 10 gallons of 1 per cent sulphuric acid and allowing this to stand for 12 hours. This extract was employed undiluted, diluted 1 to 5 and 1 to 15. The larvicidal results were not as satisfactory as those obtained above where larkspur extracts were employed. The bacterial count on manure treated with the undiluted extract was lowered somewhat, and the reaction showed a slight reduction in alkalinity due to the sulphuric acid present in the extract applied.

Hellebore.—Roots of hellebore (*Veratrum album* and *Veratrum viride*) were used both in a ground and in a powdered condition. As the following results will show, the powdered hellebore proved to be the more effective. Both 1 per cent sulphuric acid and water extracts of ground hellebore were used in cage experiments at Arlington (Table VI, Series 82, 92, 102, and 103), and the results indicate a high larvicidal action.

TABLE VI.—*Destruction of fly larvæ in horse manure. Results with ground hellebore; cage experiments, Arlington, Va., 1914.*

Series.	Treatment of 8 bushels of manure, using 10 gallons of liquid.	Flies emerged.	Apparent larvicidal effect.	Number of bacteria per 1 gram manure, calculated to dry weight.	Manure, total nitrogen.	Water extract.		
						Alkalinity, N/20 HCl per 100 c. c. (5 grams of manure).	Water-soluble nitrogen.	N as NH_3, Folin method.
		Number.	*Per cent of control average.*	*Millions.*	*Per cent.*	*C. c.*	*Per cent of total nitrogen.*	*Per cent of total nitrogen.*
82 A	Hellebore (ground), 1 pound to 10 gallons 1 per cent H_2SO_4, undiluted	10	94	423	0.526	7.15	27.76	2.85
82 B	Same as foregoing, diluted one-fifth	33	78	576	.688	10.25	24.42	2.76
87 A	Control (water only)	192	...	506	.695	11.25	28.20	1.87
87 B	Do	114	...	498	.618	15.40	27.18	2.59
87 C	Do	145	...	...	...	...	...	...
	Control average	150	...	...	...	...	...	...
92 A	Hellebore (ground), 1 pound to 10 gallons 1 per cent H_2SO_4, undiluted	35	89	...	.456	7.50	27.19	2.63
92 B	Same as foregoing, diluted one-third	18	94.3	236	.470	6.15	23.83	3.40
93 A	Control (water only)	551	...	29.8	.863	9.15	29.32	1.85
93 B	Do	333	...	12	1.45	13.25	43.72	3.25
93 C	Do	67	...	159	.905	14.15	31.05	2.87
	Control average	317	...	...	...	...	...	...
102 A	Hellebore (ground), ½ pound to 10 gallons 1 per cent H_2SO_4	315	90	...	.526	(acid).50	25.67	2.85
102 B	Do	267	92	...	.498	(acid).50	21.49	3.40
103 A	Hellebore (ground), ½ pound to 10 gallons water	58	98+	...	.568	3.50	22.70	...
103 B	Do	39	99	...	.554	6.15	28.34	1.32
106 A	Control (water only)	4,489	...	19.6	.572	4.25	25.00	2.10
106 B	Do	1,936	...	27.1	.561	4.65	26.02	1.41
	Control average	3,212	...	...	...	...	...	...

The water extract proved to be just as satisfactory as the acid extract. Table III, series 108 and 112, shows additional results from some cage experiments at New Orleans, in which the effectiveness appeared to be very low, but the results are of doubtful value for the same reasons pointed out on page 8. It is very difficult to explain why so little larvicidal effect was found in series 112 in view of the uniformly good results in other cages and open piles.

Results from the application of water extracts of ground hellebore applied to open piles are shown in Table IV, series 44 to 48, inclusive. When used at the rate of one-fourth of a pound to 10 gallons, it had a variable larvicidal action, never above 50 per cent. At the rate of three-eighths of a pound to 10 gallons the results were, on the average, somewhat higher, but none was above 70 per cent; at the rate of one-half pound to 10 gallons series 44, A and B, Table IV, showed 59 and 62 per cent action; 48, C and D, showed 44 and 62 per cent; and Table V, series 43, A and B, showed 99 per cent effectiveness.

Powdered hellebore was used in several open-pile experiments at New Orleans, and the results, as shown in Tables IV and V, indicate that the application of one-half pound per 10 gallons was uniformly favorable, the percentages varying from 88 to 99, the average of 12 open-pile experiments being 95.5 per cent. With three-eighths or one-fourth pound of powdered hellebore to 10 gallons the larvicidal effects were lower but still showed considerable action. It is evident

that the larvicidal value varies with the amount of powdered hellebore used, but when applied at the rate of one-half pound or more to 8 bushels of manure it will be efficient. It is not known how hellebore acts as a larvicide. At present no information is available as to whether it has any effect on the eggs or pupæ of the house fly.

The effects of the presence of fly maggots in a pile of manure is very strikingly shown by comparing figures 2 and 3 of Plate I. The pile treated with hellebore has remained normal in shape and appearance (Pl. I, fig. 3), while the maggots have worked the untreated pile shown (Pl. I, fig. 2), the manure being finely divided and the pile scattered by the feeding and migration of the larvæ.

The bacterial counts of manure in the cages (Table VI) treated with 1 per cent sulphuric-acid extracts of hellebore showed no bactericidal effects.

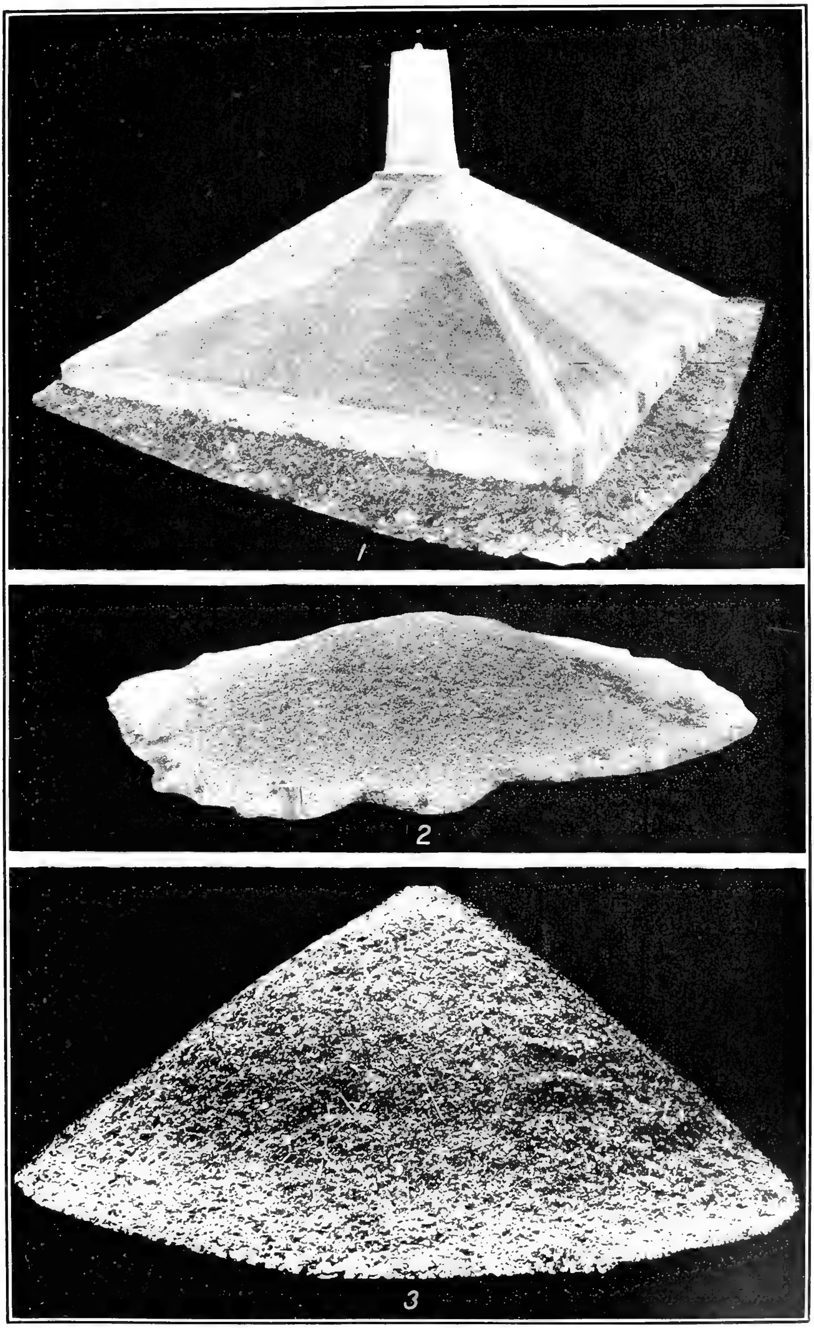
The bacterial counts of the open piles (Table IV) did not show any consistent action, either stimulating or bactericidal. During the season's work nitrites and nitrates were detected only in open-pile experiments 53, A, B, C, and D, which were treated with hellebore, and it is therefore apparent that the hellebore extract is not toxic to the nitrifying organisms in this environment.

Three series of temperatures taken daily of control piles and those treated with pyrethrum, pyridine, and hellebore further indicated that there was no permanent injury to the bacteria present in the piles treated with the last substance. In the first series the temperature was 13° below the control on the second day, but on the third day was again the same as the control. Neither of the other series showed any depression of temperature at the start, and the piles seemed to undergo a normal fermentation, indicating, as do all the data, that the treatment with hellebore does not reduce the fertilizing value of the manure.

The chemical data on both the cage and open-pile experiments show that the manure was unaffected by the hellebore treatment. When 1 per cent sulphuric-acid extracts were used in the cage experiments at Arlington, a reduction in alkalinity due to the added acid was found. It is, therefore, evident that powdered hellebore can be applied, using one-half pound to 10 gallons of water, without injuring the fertilizing value of manure as determined by chemical and bacteriological examination. Furthermore, a laboratory test has shown that hellebore readily decomposes in manure. A sample of manure treated with hellebore at the rate of one-half pound per 8 bushels when tested microscopically and colorimetrically gave positive results, but after 30 days' fermentation both were negative.

The alkaloidal content of the commercial green and white hellebore is known to vary from about 0.2 per cent to 0.9 per cent of total alkaloids.[1] In Table IV, series 53, the hellebore used was of known

[1] Data obtained from Insecticide Laboratory, Bureau of Chemistry, U. S. Department of Agriculture.



DESTRUCTION OF FLY LARVÆ IN HORSE MANURE.

FIG. 1.—Type of cage used for catching flies from treated manure piles. FIG. 2.—Settled and finely divided condition of an untreated pile, heavily infested with maggots. FIG. 3.—A hellebore-treated pile of same source and volume as figure 2. Hellebore, by preventing growth of fly maggots, prevented the disintegration of the heap. (Original.)

alkaloidal content; the two samples contained 0.25 and 0.41 per cent, respectively, and no differences in larvicidal action were evident.

The powdered hellebore used in the other experiments at New Orleans contained 11.49 per cent of ash, 1.04 per cent of total nitrogen, and about 0.2 per cent of total alkaloids. The ground hellebore used contained 29.39 per cent of ash, 1.08 per cent total nitrogen, and 0.2 per cent total alkaloids. It is therefore likely that commercial powdered hellebore of reasonable purity will be effective as a larvicide if applied as directed (p. 19).

General discussion of hellebore.—There are three plants which are popularly called hellebore, namely, *Veratrum album*, *Veratrum viride*, and *Helleborus niger*. The term "hellebore" is correctly applied only to *Helleborus niger*, which grows in Europe and is not at the present time a commercial product in this country. The white and the green are the two commercial varieties, the white being largely imported, and the green the American plant. For insecticidal work these two varieties are considered equally valuable. The American hellebore (*Veratrum viride*), called "swamp hellebore," "Indian poke," and "itch-weed," is a common plant in wet ground and grows over a considerable area of the United States. The properties of this plant are said to be similar to those of white hellebore. A number of alkaloids are claimed to have been separated from these two plants, but there is some uncertainty as to their identity and activity. Powdered hellebore, both the white and the green, is extensively used as an insecticide against the currant worm and to kill various insects around the roots of plants. Both varieties of hellebore are used in medicine to some extent.

OTHER PLANT MATERIAL.

Oxeye daisy.—Tests were made with the ground flowers of the oxeye daisy (*Chrysanthemum leucanthemum*), using 1 pound to 10 gallons of 1 per cent sulphuric acid. The material was extracted for 12 hours, and the extract was used undiluted and diluted 1 to 5. The larvicidal results were practically negative in both cases, but as the manure used in this experiment was lightly infested with larvæ and the results hardly warrant any definite conclusions. Bacteriological and chemical examinations were made of the manure treated with the undiluted extract. The bacterial count of the manure was somewhat lower than the controls, while the only noticeable change in chemical composition was a decrease in the alkalinity due to the acid in the extract added. The oxeye daisy contains a volatile oil but no alkaloid has been found.

Pyrethrum.—Pyrethrum (*Crysanthemum cinerariaefolium*) powder was tried and two results from open-pile tests at New Orleans show that solutions containing 0.5 pound per 10 gallons of water had no larvicidal action (Table V, series 52, C and D). Pyrethrum contains a volatile oil, and an alkaloid has been detected by one or two investigators.

DISCUSSION OF DIAGRAM (FIG. 1).

From the results of some open-pile experiments at New Orleans the comparative cost and larvicidal efficiency of some of the more favor-

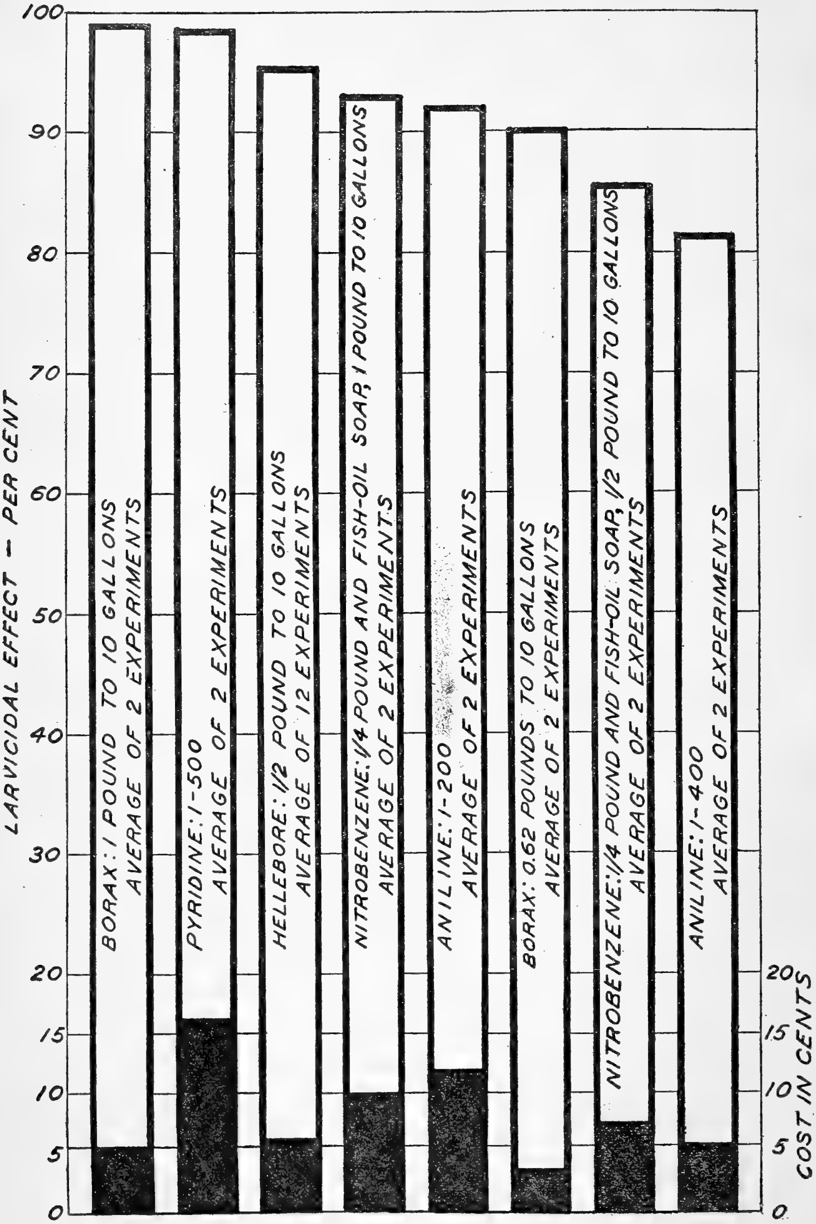


FIG. 1.—Diagram showing larvicidal effect and comparative cost of the treatment of 8 bushels of manure by various chemicals. (Original.)

able substances have been computed and are shown graphically in figure 1. Most of these calculations are based on an average of but

two experiments and are therefore to be regarded as only tentative. However, the results of the cage experiments are in general agreement with the findings as given in the diagram.

It will be noted that the highest larvicidal effect was obtained with borax, using 1 pound to 8 bushels. The least expensive treatment was that with 0.62 pound of borax, although the larvicidal action was only 90 per cent. The next cheapest was hellebore, which costs $5\frac{1}{2}$ cents for one-half pound of the powdered roots, and the average of 12 experiments showed a larvicidal action of 95.5 per cent. The hellebore treatment at the foregoing rate costs more than that with 0.62 of a pound of borax but shows a greater efficiency.

In comparing the cost we have assumed that borax can be obtained at 5 to 6 cents per pound in 100-pound lots and that hellebore can be purchased at 11 cents per pound in like amounts. The price of both is subject to considerable variation. The results in general indicate that the larvicidal action varies with the amounts used, except in the case of nitrobenzene, where the value seems to depend on the proportions of nitrobenzene and soap in the emulsion.

It will be noted that pyridine and aniline, when used in amounts sufficient to kill a high percentage of the larvæ, are quite expensive, and for this reason their use can not be considered practical.

APPLICATION OF HELLEBORE TO MANURE.

Powdered hellebore should be mixed with water at the rate of one-half pound to 10 gallons and the solution thoroughly stirred and allowed to stand for several hours in a barrel or other container. In order to obtain the most satisfactory results, the manure should be sprinkled with the foregoing solution immediately on removal from the barn. The sprinkling may be done with a watering can or similar device, using 10 gallons to 8 bushels of manure, taking care that all of the hellebore comes in contact with the manure and paying particular attention to the outer edges of the pile. In estimating the amount of solution to be employed it may be assumed that 2 bushels of manure per horse is the daily output of the stable. This is a liberal estimate, and in many stables the daily output is much less.

EFFECT OF HELLEBORE ON PLANTS AND CHICKENS.

During November, 1914, a series of tests was started at both Baton Rouge and New Orleans, La., to determine whether hellebore, when applied in considerable amounts, exerts injurious effects on plant growth. The plants grown included cabbage, lettuce, oats, turnips, radishes, potatoes, wheat, and mustard, half of each plat being fertilized with hellebore-treated manure, and the other half receiving untreated manure. At the present time no injurious

effect is noticeable from the experiments at either New Orleans or Baton Rouge.

The cooperation of Mr. George L. Tiebout, of the Louisiana Experiment Station, Baton Rouge, and of Mr. W. G. Taggart, of the Audubon Park Sugar Station, New Orleans, in connection with these tests was of great assistance.

As chickens and other farm animals are known to peck at, or consume, certain parts of manure, tests were made by Mr. E. R. Barber, placing hellebore-treated manure in coops with chickens and using as controls chickens in a coop with untreated manure. Thirty-eight one-hundredths of a pound of powdered hellebore was mixed with 4 bushels of manure and placed in one coop with four chickens, and every three days another lot of the manure similarly treated was placed in this coop. The manure which was used contained fly maggots, consequently the chickens were eager to peck through it. In addition, the chickens, in both cases, were fed on cracked corn and were given fresh water. The appearance of the chickens was noted daily, and the test has been conducted for several weeks with no apparent ill effect due to hellebore.

SUMMARY.

The larvicidal efficiency of both inorganic and organic substances was tested and bacteriological and chemical examinations of horse manure to which many of these substances were applied are reported.

The following inorganic substances were tried:

Arsenical dip.
Chlorid of lime.
Epsom salts.
Lime-sulphur.
Sulphuric acid.

Of these substances arsenical dip was the only one which when used in amounts considered practical destroyed the larvæ of the house fly. Because of its poisonous nature the use of arsenical dip as a larvicide is not recommended.

The following organic substances were tested:

Aniline.
Beta-naphthol.
Cresylic acid.
Para-dichlorobenzene.
Formaldehyde.
Nitrobenzene.
Oxalic acid.
Pyridine.

Aniline, pyridine, and nitrobenzene, when used in certain dilutions, gave satisfactory larvicidal results, but the cost precludes their use.

The larvicidal action of the following plant materials was tested:

Plant material containing saponin—

Corn cockle (*Agrostemma githago*).
Agave (*Agave lecheguilla*).

Plant material containing alkaloids—

"Black leaf 40"—tobacco extract (*Nicotiana tabacum*).
Larkspur (Delphinium).
Stramonium (*Datura stramonium*).
Hellebore (*Veratrum album* and *Veratrum viride*).

Other plant material—

Oxeye daisy (*Chrysanthemum leucanthemum*).
Pyrethrum (*Chrysanthemum cinerariaefolium*).

Powdered hellebore proved the most efficient and practical of all the substances tested.

COMPARATIVE ADVANTAGES OF BORAX AND HELLEBORE.

Borax, which was shown in Bulletin No. 118 to be an effective larvicide, is obtainable in all parts of the country, and the cost of treating manure at the rate of 0.62 pound of borax per 8 bushels is 0.42 cent per bushel.

Powdered hellebore, using one-half pound to 10 gallons of water and applying this to 8 bushels of manure, is also an effective larvicide and exerts no injurious action on the fertilizing value of the manure as determined by bacteriological and chemical analyses, and no injurious action on plants has been detected in any of the field tests. Hellebore is used as an insecticide and is obtainable in most cities and agricultural districts. The cost of this treatment is 0.69 cent per bushel of manure.

While borax may be applied to manure at the foregoing rate and the treated manure may be added to the soil at the rate of 15 tons to the acre without injuring vegetation, nevertheless excessive quantities of borax may be applied to manure through carelessness, and injury to vegetation may in consequence result. In the light of this year's experiments it seems advisable to recommend borax as a larvicide for the treatment of outhouses, refuse piles, and all other places where flies may deposit eggs. However, on account of the possible carelessness previously mentioned, and because large quantities of manure are sometimes used by truck growers, it seems best to guard against possible injury to vegetation by recommending powdered hellebore for the treatment of manure, since no injury can arise from the use of excessive quantities, as it is entirely decomposed in the course of the fermentation of the manure.

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